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REMARKS

Reconsideration of the Final Office Action mailed March 12, 2004, (hereinafter "instant Office Action"), entry of the foregoing amendments, withdrawal of the rejection of claims 21-27, 32 and 33 and the withdrawal of the objection to claim 32 are respectfully requested.

In the instant Office Action, claims 1-88 are listed as pending, claims 1-20, 28-31 and 34-88 are withdrawn from consideration, claims 21-27, 32 and 33 are listed as rejected and claim 32 is objected to.

Applicants gratefully acknowledge that the Examiner has withdrawn her objections to the specification alleging that it contains "an embedded hyperlink and/or other form of browser-executable code", such as on page 23, line 14 and elsewhere, the minor informality of "anaology" being misspelled on page 14, line 28 and the minor informalities in claims 21-24, 26 and 27.

Applicants also gratefully acknowledge that the Examiner has withdrawn the rejection of claim 32 under 35 U.S.C. §112, first paragraph, with respect to the term "the ligand".

The Examiner has objected to Claim 32 because it fails to end in a period. Applicants have amended claim 32 to add a period at its end.

The Examiner has maintained the rejection of claims 21-27, 32 and 33 under 35 U.S.C. §112, first paragraph, alleging that the specification, while being enabling for the atomic coordinates for residues 802-1124 of Tie-2 and Inhibitor III complex, does not reasonably provide enablement for the atomic coordinates of an unbound version of a Tie-2 polypeptide or atomic coordinates of the complete polypeptide of Tie-2 and Inhibitor III complex. The Examiner alleges that the invention as presently stated in claim 21 encompasses these additional sets of atomic coordinates, but that they are not included in the specification which consequently causes a lack of scope of enablement of the instant invention for one of ordinary skill in the art. Applicants respectfully traverse this rejection. Applicants maintain the arguments that were presented in the Reply mailed December 23, 2003.

According to M.P.E.P. §2164.01, "Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination as to whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the art to make and use the claimed invention." Applicants respectfully point out that Claim 21 is a method claim and step (a) is directed to obtaining the

atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of a Tie-2 protein. Applicants have taught how to obtain the atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of a Tie-2 protein, *inter alia*, at page 11, lines 3-8 and page 26, lines 14-16. Step (b) of claim 21 is using said atomic coordinates to define the active subsite of Tie-2. Applicants have shown how to solve the crystal structure of a polypeptide comprising the catalytic domain of a Tie-2 protein on page 48, line 28 to page 49, line 14 and page 50, lines 1-27 and how to define the active subsites on page 50, lines 10-19, using various computer programs. On page 51, lines 1-7 Applicants teach inhibitor docking.

Step (c) of claim 21 is identifying a compound which binds to one or more active subsites wherein the compound which binds to the active subsite or sites is an inhibitor of a Tie-2 protein. Applicants teach methods of identifying a compound which bind to one or more active subsites on page 29, line 11 to page 31, line 19.

In Example 2, Applicants have exemplified all steps of claim 21 by identifying a compound which is an inhibitor of Tie-2 by obtaining the atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of a Tie-2 protein, using these atomic coordinates to define the active subsites of Tie-2 and identifying a compound which binds to one or more active subsites and inhibit the Tie-2 protein.

The Examiner states that Applicants have disclosed information to enable one skilled in the art to make a diphosphorylated Tie-2 protein crystal of the space group $P2_12_12_1$ with unit cell dimension a=54.320Å, b=75.872 Å, c=78.143 Å, and $\alpha=\beta=\gamma=90.0°$, a catalytically inactive mutant of human Tie-2, and the crystal structure of three other crystals of Tie-2 ligand complexes, but the specification does not reasonably provide enablement for finding atomic coordinates of an unbound Tie-2 polypeptide as well as an entire Tie-2 polypeptide and Inhibitor III complex as encompassed in claim 21. Claim 21 is directed to a method of identifying compounds which are inhibitors of a Tie-2 protein, the first step of which is obtaining the atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of a Tie-2 protein. The polypeptide must at a minimum include the catalytic domain of the Tie-2 protein, but may include additional amino acid residues. The catalytic domain of Tie-2 is defined on page 10, lines 3-6 as being "...defined by amino acid residues from about residue 828 to about residue 985 of SEQ ID NO: 1, with residues 828-840, 853-855, 872, 873, 876, 879, 880, 885-888, 900, 902-909, 912, 954, 955, 960, 964, 968-971, and 980-985 included in the catalytic domain."

Applicants have enabled finding atomic coordinates of an unbound Tie-2 polypeptide as well as an entire Tie-2 polypeptide and Inhibitor III complex containing the catalytic domain of a Tie-2 protein.

The Examiner states "[d]ue to the unpredictability and difficulty of crystallizing proteins, it is unlikely that one of skill in the art would be able to make another crystal relying solely on the information for the crystal disclosed in the specification without undue experimentation." Applicants maintain that the Examiner needs to show specific reasons why other embodiments within the full scope of claim 21 would not work, rather than merely alleging the "unpredictability" of crystallizing proteins. "A general allegation of 'unpredictability in the art' is not a sufficient reason to support a rejection for lack of adequate written description." M.P.E.P. 2163.04. In fact, crystallizing proteins is not unpredictable nor does it entail undue experimentation.

The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art: Ansul Co. v. Uniroyal, Inc., 4 F.2d 872 (2d Cir. 1971). "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. The factors to be considered have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in that art, the predictability or unpredictability of the art and the breadth of the claims." In re Rainer, 52 CCPA 1593, 347 F.2d 574, 146 USPQ 218 (1965); In re Colonianni, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977).

The instant specification teaches crystallization conditions for diphosphorylated Tie-2 802-1124 on page 48, Tie-2 (D964N) 802-1124 (SEQ ID NO 1)on page 49 and for Tie-2 (D964N) 802-11234 (SEQ ID NO 2) on page 51 of the instant application. Further, Table II on pages 53-56 lists crystallization conditions for Tie-2/inhibitor complexes. The amount of experimentation required to utilize the instant invention is routine in the field of protein crystallography, and, thus, is not undue. To crystallize a protein, one makes the protein, brings it

to a suitable concentration and mixes it with a precipitating agent. If a crystal does not form, one of ordinary skill in the art would know to try a different precipitating agent or alter some of the other factors influencing protein crystal growth, such as pH or temperature. One skilled in the art would know that in order to crystallize proteins, one methodically adjusts the ratio of solvents used or the experimental conditions. In fact, these routine, repetitive procedures can be done by robots or other automated machinery.

Crystallization of Membrane Proteins, ed. Hartmut Michel (Boca Raton: CRC Press, 1991), serves as a reference with regard to crystallizing proteins. Pages 37 and 38 are attached as Exhibit A for the Examiner's convenience. In Chapter 1, Alexander McPherson discusses the variables important to crystal growth. McPherson lists salts and organic solvents used in the crystallization of proteins, as well as factors influencing protein crystal growth. One of ordinary skill in the art would be familiar with the different precipitating agents or crystallization modalities to try in the event that the first attempt to crystallize a protein did not work. McPherson points out that one may need to adjust the variable parameters in order to successfully obtain a crystal. As stated in M.P.E.P. §2164.01, "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation". *In re Certain Limited-Charge Cell Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), aff'd sub nom. *Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985). As illustrated by McPherson, crystallizing proteins may require fine tuning reagents and/or conditions but one skilled in the art knows how to adjust solvents, precipitants or other factors in order to improve the crystallization conditions and the likelihood of obtaining the crystal. Applicants have enabled claims 21-27, 32 and 33 because not only have Applicants provided crystallization conditions, *inter alia*, on pages 53-56, but the knowledge needed regarding how to regulate the crystallization conditions is generally available, as evidenced by the 1991 publication of *Crystallization of Membrane Proteins*, as well as other numerous references. "The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public." *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94, (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann*

Maschinenfabrik GMBH v American Hoist & Derrick Co., 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Thus, through examples and teachings throughout the instant specification Applicants have enabled the instant invention. Applicants have taught all of the steps of claim 21 and enabled others to utilize the claimed method to identify compounds which are inhibitors of a Tie-2 protein.

Based upon the foregoing, the rejection of claims 21-27, 32 and 33 under 35 U.S.C. §112, first paragraph, for lack of scope enablement is obviated and should be withdrawn.

The Examiner has rejected Claims 21-27, 32 and 33 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the invention was filed, had possession of the claimed invention. The Examiner alleges that "due to the open claim language of 'comprises' in claim 21, this claim is directed to encompass amino acid sequences that do not meet the written description provision of 35 U.S.C. §112, first paragraph." Applicants respectfully traverse this rejection. Applicants maintain the arguments presented in the Reply filed December 23, 2003.

The Examiner cites *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111 in which the Board held that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." Applicants' written description clearly conveys that Applicants had possession of the instant invention at the time of filing. Applicants described the claimed invention with limitations using words and formulas. Applicants provide the atomic coordinates for the crystals, which is the equivalent of drawing a structure.

One of ordinary skill in the art of protein crystallography would understand the written description and claims as filed by Applicants. The application as originally filed provided adequate written description for the claims as originally filed. Applicants have crystallized approximately 95% of the cytoplasmic domain of Tie-2. By crystallizing the catalytic domain Applicants have defined the important part of the Tie-2 protein. Only 30 amino acids, the sequence of which are known, are missing from the N-terminus of Applicants' crystal. Applicants respectfully direct the Examiner's attention to M.P.E.P. §2163, which states:

> Possession may be shown in many ways. For example, possession may be shown by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. See, *e.g.*, *Purdue Pharma L.P. v. Fauling Inc.*, 230 F.3d 1320, 1323, 57 USPQ2d 1481, 1483 (Fed. Cir. 2000).

Applicants have reduced the instant invention to practice. Specifically, Applicants have shown how to solve the crystal structure of a polypeptide comprising the catalytic domain of a Tie-2 protein on page 48, line 28 to page 49, line 14 and page 50, lines 1-27 and how to define the active subsites on page 50, lines 10-19, using various computer programs. On page 51, lines 1-7 Applicants teach inhibitor docking. In Example 2, Applicants have demonstrated identifying a compound which is an inhibitor of Tie-2 by obtaining the atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of a Tie-2 protein, using these atomic coordinates to define the active subsites of Tie-2 and identifying a compound which binds to one or more active subsites and inhibits the Tie-2 protein. Applicants have shown sufficient examples to demonstrate that the instant method works. Applicants' written description, through text, formulas and working examples, convey that Applicants had possession of the full scope of the invention at the time the instant application was filed.

There is no difference between claim 21 and a method claim directed to use of a compound described by a chemical genus, or even a composition of matter claim directed to a chemical genus, in that an Applicant is not required to provide a working example of every possible embodiment covered by the claim. Instead, for a claim directed to a chemical genus, examples representative of the genus are considered sufficient. As Applicants stated in the Reply filed December 23, 2003, there is no a requirement as to how many working examples must be provided in a patent application. As stated above, in the instant application Applicants have shown how to solve the crystal structure of a polypeptide comprising the catalytic domain of a Tie-2 protein, how to define the active subsites and taught inhibitor docking, all leading to identifying further inhibitors. On pages 53-57 Applicants have provided crystallization conditions for Tie-2/inhibitor complexes. Applicants have provided a written description for the instant invention.

The Examiner cites *Fiers v. Revel*, 25 USPQ2d 1601, 1601 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 to support her allegation that "[a]dequate written description requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it. The nucleic acid itself is required." These cases are not on point. The claim at issue in *Fiers* was directed to a DNA. In *Fiers*, the Board held that "[a]n adequate written description of a DNA requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself." (emphasis added). Further, the Board in *Fiers* held, "[i]f a conception of a DNA requires a precise definition, such as by structure, formula, chemical name or physical properties, as we have held, then a description also requires that degree of specificity." Claim 21 in the instant application is directed to a method. Applicants have described the steps of the method and taught how to perform the steps of the method. As discussed above in the response to the Examiner's citation of *Vas-Cath*, Applicants provide the atomic coordinates for the crystals, which is the equivalent of drawing a chemical structure. Thus, Applicants have provided specific written description of the invention.

With respect to *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016,* the case involved production of EPO and whether the defendant had disclosed the best mode and had enabled the claims. There was no question as to whether the defendant had provided adequate written description. The only reference to satisfying the written description requirement is in regard to patent applicants placing microorganism samples in a public depository when such a sample is necessary to carry out the claimed invention. Such a deposit is considered to satisfy the *enablement* requirement of 35 U.S.C. §112, when a written description alone would not place the invention in the hands of the public and physical possession of a unique biological material is required. Neither of these issues is relevant to the question of whether Applicants have satisfied the written description requirement in the instant application. The claim at issue in *Amgen* is directed to a DNA sequence. Claim 21 in the instant application is directed to a method of identifying a compound which is an inhibitor of Tie-2.

The Examiner also cites *University of California v. Eli Lilly and Co.*, 43 USPQ2d 1398, 1404, 1405 with regard to the lack of written description. In *University of California v. Eli Lilly and Co.* the Board held "[i]n claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can

distinguish such a formula from others and can identify many of the species that the claims encompass." The Board further held "[a] description of a genus cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. This is analogous to enablement of a genus under §112, P1, by showing the enablement of a representative number of species within the genus. See *Angstadt*, 537 F.2d at 502-03, 190 USPQ (BNA) at 218 (decided that applicants 'are not required to disclose every species encompassed by their claims even in an unpredictable art' and that the disclosure of forty working examples sufficiently described subject matter of claims directed to a generic process);...". This case is not on point because it deals with DNA, whereas the instant application is directed to a method of identifying a compound which is an inhibitor of Tie-2.

The Examiner states that because the method contains reference to a polypeptide the same general principals of *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.* and *University of California v. Eli Lilly and Co.* apply. Applicants respectfully point out that in each case the claims in question involved DNA, whereas claim 21 of the instant application is directed to a method. The polypeptide referred to in the claim is not the invention. The invention is the method. Applicants have satisfied the written description requirements for the method of claim 21 by showing possession of the invention, describing the invention in detail and reducing it to practice.

Based upon the foregoing, the rejection of Claims 21-27, 32 and 33 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time of the invention was filed, had possession of the claimed invention, is obviated and should be withdrawn.

The Examiner has rejected claims 21-27, 32 and 33 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The Examiner alleges that claims 21-25 are vague and indefinite due to the unclarity of citing an abbreviation, such as Tie-2. Applicants respectfully traverse this rejection. In the Reply filed December 23, 2003 Applicants submitted as Exhibit A a copy of Shawver, Laura K. et al, DDT, Vol. 2, No. 2, February 1997, "Receptor

tyrosine kinases as targets for inhibition of angiogenesis" as evidence that the term "Tie-2" is a well known term of art and was known prior to March 22, 2001, the filing date of the instant application. The Examiner stated that "[t]his is found unpersuasive as a single publication is evidence that a term is "known" in the art, but does not support the "well known" character of such a term. A well known term would be evidenced by a textbook citation of the term or a review article, or alternatively, at least a few publications from difference sources. Applicants respectfully point out that the paper by Shawver et al. is a review article which appeared in the publication Drug Discovery Today, and thus satisfies the requirement sought by the Examiner that a well known term would be evidenced by citation of the term in a review article.

Based upon the foregoing, the rejection of 21-27, 32 and 33 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention, is obviated and should be withdrawn.

The Examiner has rejected claims 21, 22 and 26 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)). Applicants respectfully traverse this rejection.

The Examiner states that Chen et al. describe a method for identifying an agent that diminishes the activity of a protein (col. 4, lines 56-60). In fact, Chen et al. describes a method for identifying a drug that affects the ability of STAT to *induce expression of a gene* under the control of a promoter containing a binding site for STAT, whereas the instant application is directed to a method of identifying a compound which is an inhibitor of a Tie-2 protein. Chen et al. involves inducing gene expression whereas gene expression is not a part of the instant invention. Chen et al. also do not teach or suggest the step of obtaining the atomic coordinates of a Tie-2 protein.

The Examiner states "[t]he MPEP indicates that descriptive material unable to exhibit any functional interrelationship with the way in which computing processes are performed does not constitute a statutory process, machine, manufacture or composition (MPEP § 2106, section VI)." Applicants respectfully point out that claim 21 is directed to a method, not the atomic coordinates themselves. The Examiner further states "[d]ue to the fact that the coordinate data set derived from the crystal structure of the Tie-2 protein or Tie-2/Inhibitor III complex to develop three-dimensional models in the instant case are merely stored so as to be read or outputted by a computer without creating functional interrelationship, either a part of the stored

data or as part of the computing processes performed by the computer, this descriptive material alone does not impart functionality either to the data as structured, or to the computer". Applicants respectfully point out that the claims are directed a method that uses the atomic coordinates which define the active subsites of Tie-2 to define the structure of the Tie-2 protein. The coordinates are equivalent to a drawn chemical structure. Without the coordinates one cannot envision the structure of the Tie-2 protein or identify compounds which will bind to the specific active subsites. The atomic coordinates are functionally related to both the crystal polypeptide, from which they are obtained, and the compound which is identified based upon the atomic coordinates. Without the atomic coordinates, one would not be able to design a compound made to inhibit the Tie-2 protein. The details of docking results depend intimately from the functional results computed from these coordinates. Using the atomic coordinates in this way is functionally equivalent to ascertaining the structure of an organic compound and using it as a basis for making further analogs. The atomic coordinates are not separable from the method of designing the inhibitor.

Based upon the foregoing, the rejection of claims 21, 22 and 26 under 35 U.S.C. §103(a) over Chen et al. in view of *In re Gulack* is obviated and should be withdrawn.

The Examiner has rejected claims 21-27 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)), *In re Best* (195 USPQ 430) and *In re Fitzgerald* (205 USPQ 594) and Ziegler (P/N 5,447,860). Applicants respectfully traverse this rejection and maintain the arguments presented in the Reply filed December 23, 2003.

As argued above in the rejection of claims 21, 22 and 26 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)), Chen et al. describes a method for identifying a drug that affects the ability of STAT to induce expression of a gene under the control of a promoter containing a binding site for STAT whereas the instant application is directed to a method of identifying a compound which is an inhibitor of a Tie-2 protein. Also as argued above, the atomic coordinates do have a functional relationship to the invention. Without said coordinates, one would be unable to use the instant invention, i.e. identify compounds which act as inhibitors of Tie-2.

With respect to Ziegler (P/N 5,447,860), which the Examiner states "...the sequences of ork (as stated by Ziegler) and Tie-2 (as stated in the instant invention) appear to be the same...", Applicants respectfully point out that the context of Ziegler makes clear that Ziegler refers to the biological ligand of Tie that binds to the extracellular domain, not the small molecule ligands that bind to the catalytic domain of Tie-2. Step (a0) of claim 21 is directed to obtaining the atomic coordinates of a crystal of a polypeptide comprising the catalytic domain of Tie-2. The catalytic domain is inside the cell, as opposed to the extracellular domain, which is outside the cell. Ziegler does not include the catalytic domain of Tie-2. Applicants further note that Ziegler teaches that ork is not Tie. Ziegler provides a novel protein kinase. It teaches and suggests nothing about identifying compounds to inhibit said protein kinase, much less doing it using crystal coordinates.

With respect to *In re Best* (195 USPQ 430) and *In re Fitzgerald* (205 USPQ 594), neither Ziegler nor Chen et al., alone or in combination, teach or suggest Applicants' method of identifying inhibitors of Tie-2 proteins. Chen et al. is limited to STAT protein. Neither reference teaches or suggests using atomic coordinates to define the active subsite of Tie-2. One would not be motivated to look to these references to arrive at Applicants' invention.

Based upon the foregoing, the rejection claims 21-27 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)), *In re Best* (195 USPW 430) and *In re Fitzgerald* (205 USPQ 594) and Ziegler (P/N 5,447,860) is obviated and should be withdrawn.

The Examiner has rejected claims 21-27 under 35 U.S.C. §103(a) as being unpatentable over Chen et al. (P/N 6,160,092) in view of Vikkula et al. (Cell, 1996, Volume 87, pages 1181-1190) and *In re Best* (195 USPQ 430) and *In re Fitzgerald* (205 USPQ 594). Applicants respectfully traverse this rejection.

The Examiner has not established a *prima facie* case of obviousness in any of the foregoing rejections. In order to establish a *prima facie* case of obviousness, first there must be some suggestion or motivation to modify the reference cited by the Examiner. Second, there must be a reasonable expectation of success. One would not look to Vikkula et al., which describes mutations in the kinase domain of Tie-2 that result in increased activity of Tie-2 and that an activating mutation in Tie-2 causes venous malformations, for guidance on a method to identify compounds that inhibit Tie-2 proteins. Further, the prior art reference (or references

when combined) must teach or suggest all of the claim limitations. Neither Vikkula et al. nor Chen et al. alone or in combination teach or suggest a method of identifying compounds that inhibit a Tie-2 protein using crystal coordinates to define the active subsites of Tie-2 and identifying a compound which binds to one or more of these active subsites.

To establish a *prima facie* case of obviousness, the invention must be considered as a whole, there must be some suggestion or motivation to modify the reference, the reference must teach or suggest all of the claim limitations and there must be a reasonable chance of success. The Examiner has not provided any motivation to modify Vikkula et al. Further, Vikkula et al. does not teach or suggest all of the limitations of Applicants' claims. As stated in M.P.E.P. 2143.03, "To establish prima facie obviousness of a claimed invention, all of the claim limitations must be taught or suggested by the prior art." In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Even if Vikkula et al. and Chen et al. were combined, they do not teach or suggest a method of identifying compounds that inhibit a Tie-2 protein using crystal coordinates to define the active subsites of Tie-2 and identifying a compound which binds to one or more of these active subsites.

No such motivation or suggestion exists in Vikkula et al. When the prior art fails to suggest the claimed invention as a whole, as it does here, any reconstruction of the prior art to obtain that invention necessarily and inevitably requires impermissible hindsight.

The same arguments as made above in response to the rejections of claims 21, 22 and 26 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)) and claims 21-27 under 35 U.S.C. §103(a) as allegedly being unpatentable over Chen et al (P/N 6,160,092) in view of *In re Gulack* (703 F.2d 1381, 1385, 217 USPQ 401, 404 (Fed. Cir. 1983)), *In re Best* (195 USPQ 430) and *In re Fitzgerald* (205 USPQ 594) apply to this rejection as well.

With respect to Vikkula et al., Applicants respectfully point out that the claims under rejection are directed to a method of identifying a compound which is an inhibitor of a Tie-2 protein. Said method is comprised of specific steps that are listed in claim 21. Vikkula et al., on the other hand, discloses that mutations in the kinase domain of Tie-2 result in increased activity of Tie-2 and that an activating mutation in Tie-2 causes venous malformations. Vikkula et al. does not teach or suggest Applicants' method to identify a compound that inhibits Tie-2.

The Examiner states that "Vikkula et al. describes a Gen Bank accession number L06139 (see GenBank reference with both nucleic acid and polypeptide translation) which is nucleic acid

sequence of human Tie-2 protein (page 1183, col. 2, first paragraph) that is 100% identical to the residues 802-1124 of the sequence in the instant invention." Nonetheless, Vikkula et al. alone or in combination with Chen et al (P/N 6,160,092) do not teach or suggest Applicants' method of obtaining atomic coordinates and using said atomic coordinates to obtain a compound that is an inhibitor of a Tie-2 protein.

Based upon the foregoing, Applicants believe the rejection of claims 21-27 under 35 U.S.C. §103(a) as being unpatentable over Chen et al. (P/N 6,160,092) in view of Vikkula et al. (Cell, 1996, Volume 87, pages 1181-1190) and *In re Best* (195 USPQ 430) and *In re Fitzgerald* (205 USPQ 594) is obviated and should be withdrawn.

No fees are due for the instant amendment since the total number of claims after entry of the amendments hereinabove is not more than the total number of claims that Applicants have paid for to date.

Based upon the foregoing, Applicants believe that claims 21-27, 32 and 33 are in condition for allowance. Prompt and favorable action is earnestly solicited.

If the Examiner believes that a telephone conference would advance the condition of the instant application for allowance, Applicants invite the Examiner to call Applicants' agent at the number noted below.

Respectfully submitted,

Date: July 7, 2004

Gayle O'Brien

Gayle B. O'Brien
Agent for Applicants
Reg. No. 48,812

Abbott Bioresearch Center
100 Research Drive
Worcester, MA 01605
(508) 688-8053

Exhibit A

Crystallization of Membrane Proteins

Editor
Hartmut Michel
Director
Department of Molecular Membrane Biology
Max-Planck-Institute of Biophysics
Frankfurt/Main, Germany

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Boca Raton Ann Arbor Boston

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may occur within a few hours or a few days. It seldom requires more than 3 weeks. Thus evaluation of results can be made without undue demands on patience. It should be noted that protein-PEG solutions are excellent media on which to grow microbes, particularly molds, and if crystallizatin is being attempted at room temperature or over extended periods of time, then some retardant such as azide (commonly 0.1%) must be included in the protein solutions.

Since PEG solutions are not volatile, PEG must be used like salt and equilibrated with the protein by dialysis, slow mixing, or vapor equilibration. This latter procedure, utilizing either 10 μl hanging drops over 0.5 ml reservoirs or 20 μl drops on multidepression glass plates in a sealed chamber, has proven the most popular. The author has found that when the reservoir concentration is in the range of 5 to 12%, the protein solution to be equilibrated should be at an initial concentration of about half. That is conveniently obtained by adding 10 μl of the reservoir to 10 μl of the protein solution. When the final PEG concentration to be obtained is much higher than 12%, it is advisable to start the protein equilibrating at no more than 4 to 5% below the final value. This reduces unnecessary time lags during which the protein might denature.

Crystallization of proteins with PEG has proven more successful when the ionic strength is low. It is quite difficult when ionic strength is high. Good buffer conditions in the neutral range are, for example, 10 to 40 m*M* Tris or cacodylate buffer. If crystallization proceeds too rapidly, addition of some neutral salt may be used to slow growth and better effect crystal form. PEGs are useful over the entire pH range and over a broad temperature range and show no anomalous effects in repsonse to either. PEG appears to be an excellent crystallization agent over the whole spectrum of proteins, although in specific cases other precipitants may be superior.

XIII. FACTORS INFLUENCING PROTEIN CRYSTAL GROWTH

Table 4 lists physical, chemical, and biological variables that may influence to a greater or lesser extent the crystallization of proteins. The difficulty in properly arriving at a just assignment of importance for each factor is substantial for several reasons. Every protein is different in its properties and, surprisingly perhaps, this applies even to proteins that differ by no more than one or just a few amino acids. There are even cases where the identical protein prepared by different procedures or at different times may show significant variations. In addition, each factor may differ considerably in importance for individual proteins. α-Amylase, for example, is strikingly sensitive to temperature change, while concanavalins A and B show little or no variation in crystallization properties as a function of temperature.

Because each protein is unique, there are few means available to predict in advance what specific values of a variable, or sets of conditions might be most profitably explored. Finally, the various parameters under one's control are not independent of one another and their interrelations may be complex and difficult to discern. It is, therefore, difficult to elaborate a rational set of guidelines relating to physical factors or ingredients in the mother liquor that can increase the probability of success in crystallizing a particular protein. The specific components and conditions must be painstakingly sought and refined for each macromolecule.

As already noted, temperature may be of great importance or it may have little bearing at all. In general, it is wise to initially duplicate all crystallization trials and conduct parallel investigations at 4 and at 25°C. Even if no crystals are observed at either temperature, differences in the precipitation behavior of the protein with different precipitants and with various effector molecules may give some indication as to whether temperature will play an important role. If crystals are observed to grow at one temperature and not, under otherwise identical conditions, at the other, then further refinement of this variable is necessary. This

TABLE 4
Factors Affecting Protein Crystal Growth

1. pH
2. Ionic strength
3. Temperature
4. Concentration of precipitant
5. Concentration of macromolecule
6. Purity of macromolecules
7. Additives, effectors, and ligands
8. Organism source of macromolecule
9. Substrates, coenzymes, inhibitors
10. Reducing or oxidizing environment
11. Metal ions
12. Rate of equilibration
13. Surfactants or detergents
14. Gravity
15. Vibrations and sound
16. Volume of crystallization sample
17. Presence of amorphous material
18. Surfaces of crystallization vessels
19. Proteolysis
20. Contamination by microbes
21. Pressure
22. Electric and magnetic fields
23. Handling by investigator

is accomplisheed by conducting the trials under the previously successful conditions over a range of temperatures centered on the one that initially yielded crystals.

The only general rules with regard to temperature seem to be that proteins in a high salt solution are more soluble in colder than in a warmer temperature. Proteins, however, generally precipitate or crystallize from a lower concentration of PEG, MPD, or organic solvent at cold temperature than at warmer temperature. One must remember, however, that diffusion rates are less and equilibration slower at cold temperature than higher temperature, so that the times for precipitation or crystal formation may be longer in the cold.

After precipitant concentration, the next most important variable in protein crystal growth appears to be pH. This follows logically since the charge character of a protein and all of its attendant physical and chemical consequences are intimately dependent on the ionization state of the amino acids that comprise the macromolecule. Not only does the net charge on the protein change with pH, but the distribution of those charges, the dipole moment of the protein, its conformation, and in many cases its aggregation state. Thus an investigation of the behavior of a specific protein as a function of pH is perhaps the single most essential analysis that should be carried out in attempting to crystallize the macromolecule.

As with temperature, the procedure to follow is to first conduct parallel crystallization trials at course intervals over a broad pH range and then repeat the trials over a finer grid of values in the neighborhoods of those that initially showed promise. The only limitations on the breadth of the initial range screened are the points at which the protein begins to lose activity and show indications of denaturation. In refining the pH for optimal growth, it should be recalled that the difference between amorphous precipitate, microcrystals, and large single crystals may be only a few tenths of a pH unit.[105]

In addition to adjusting pH for the optimization of crystal size, it is sometimes also useful to explore variation of pH as a means of altering the habit or morphology of a crystalline protein. This is occasionally necessary if the initial crystal form is not amenable to analysis because it grows as fine needles or flat, thin plates or demonstrates some other unfavorable tendency such as striation or twinning.